EUROBIOFILMS 2011

FLUXION

BIOFILMS 2.0

The Next Generation Toolkit for Microbial Analysis

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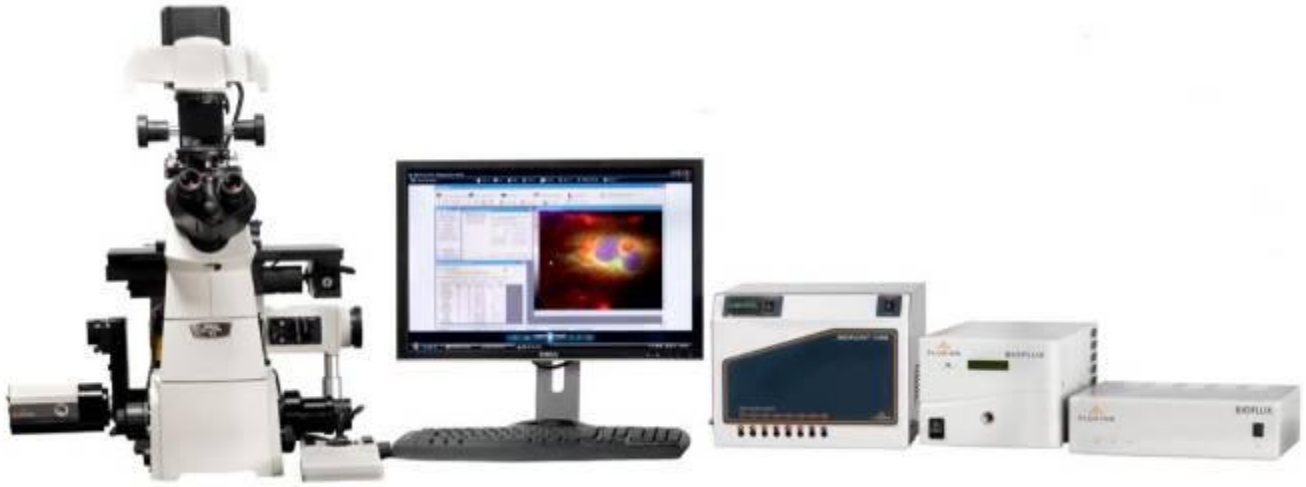
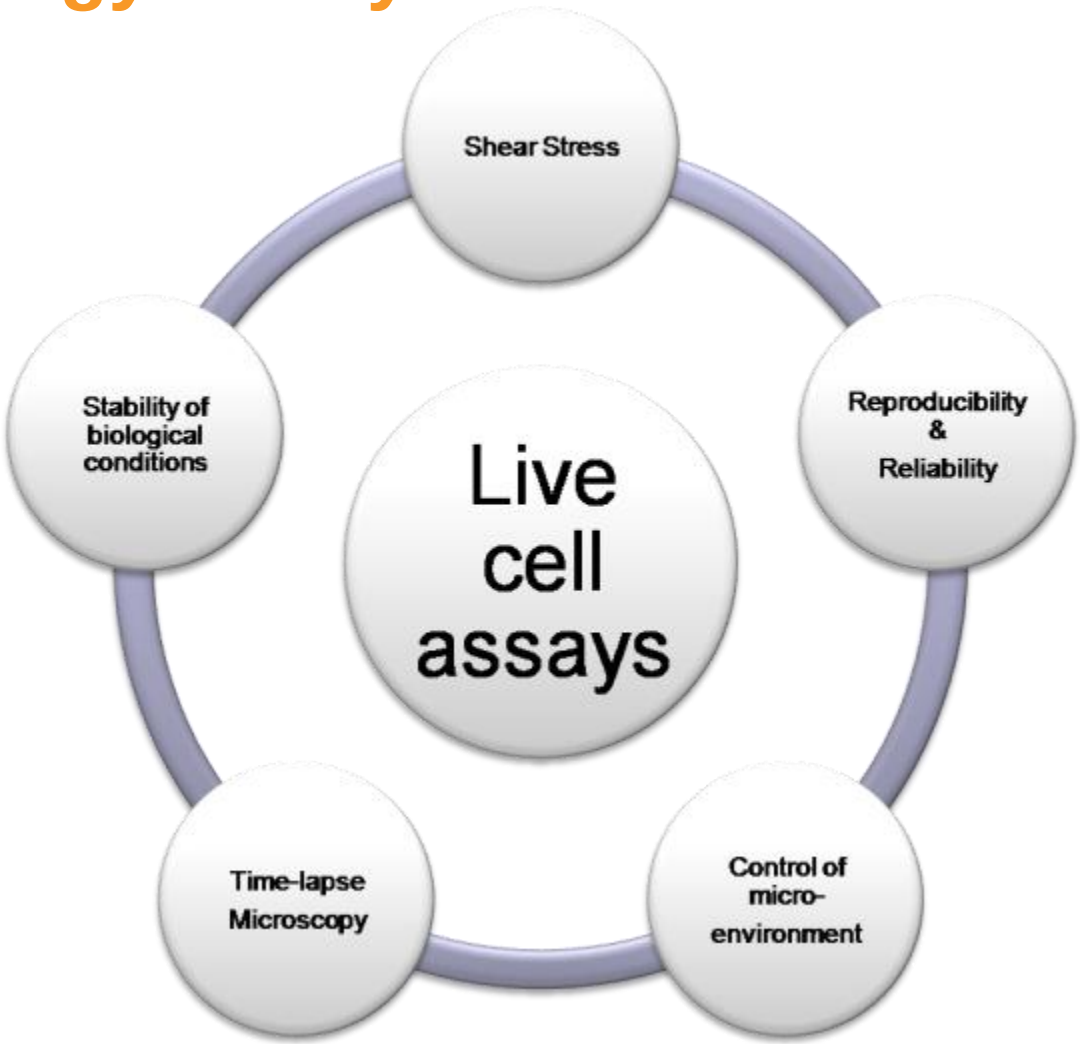
Presentation Overview

In this presentation, you will be presented with…

1. Critical parameters for microbiology assays delivered with microfluidics
2. The basic principles of operation for BioFlux
3. A review of current microbiology related applications
4. The typical configurations of a BioFlux System



Critical Variables to Control For in Microbiology Assays



BioFlux for Microbiology

Shear

- Stimulation
  - Biofilm formation
  - Microbial attachment
- Adhesion strength
  - Pathogenesis
  - Industrial applications

- Biological Niche
  - Blood vessels, catheter, UT, GI,
  - Respiratory tract, oral cavity

Flow

- Standardization of assay conditions
  - Assay reproducibility
  - Continuous conditions
  - Sampling temporal events
  - Wound healing assays

- Host-Pathogen

  Introduction of bacteria, fungi or parasites to host cells for live cell biology

BioFlux Provides The Control Necessary to Run Physiologically Relevant Assays

- Shear stress control
- Temperature control
- Atmospheric control
- Controlled micro-compartmentalization of channels

The BioFlux Solution

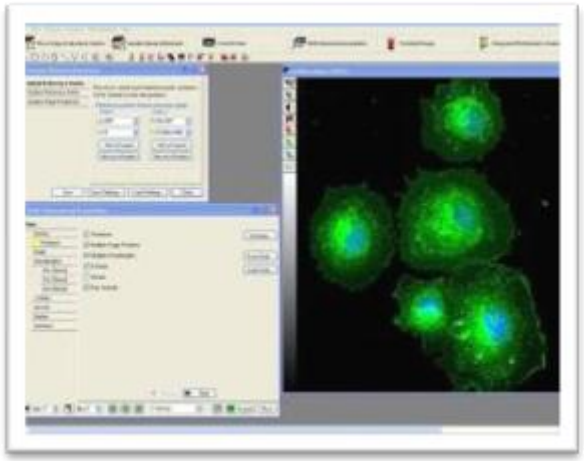
- BioFlux **integrates** the **ease of use** and **throughput** of a well plate assay with the **biological relevance** of a flow enabled experiment under shear stress.
  - Applies constant shear
  - Can easily run up to 96 assays at a time
  - Simple setup and disposal without the need for sterilization of any components
  - Simple automation of protocols and software for analysis
  - Cost effective use of media and cells
    - (<3 mls of media/well/day)
    - Only requires 50,000 cells /expt for creating a monolayer
    - Only requires about 50uL of drug /expt
  - Rapid, reproducible and precise flow control
  - Can run experiments under hypoxic conditions

BioFlux System – Key Components

Pneumatic controller

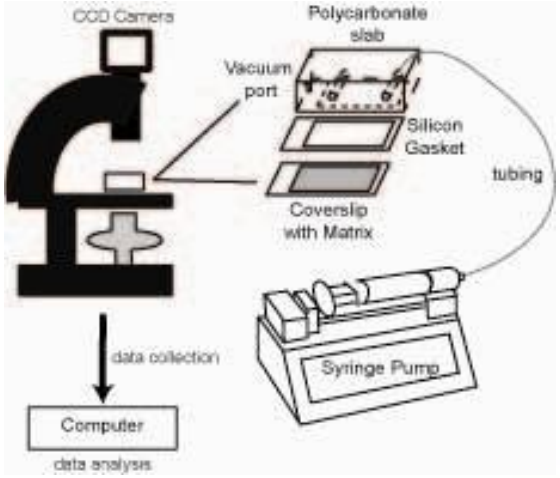
Well Plate Microfluidic™ Devices

Integrated microscopy automation



Data acquisition and analysis software

BioFlux Provides What A Traditional Parallel Plate Flow Chamber Can't…..Scalability



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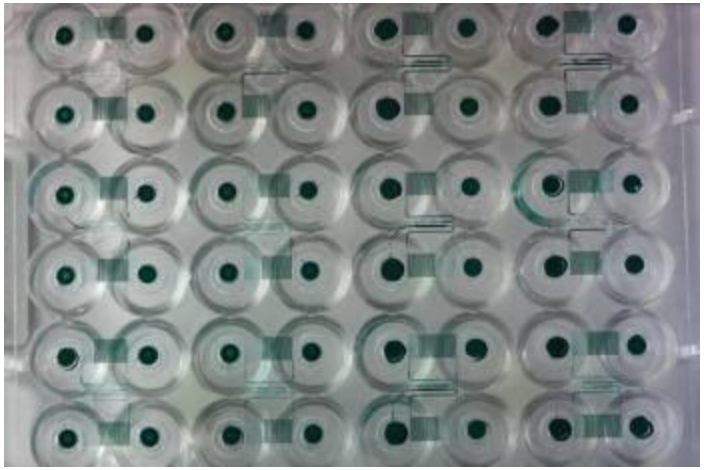
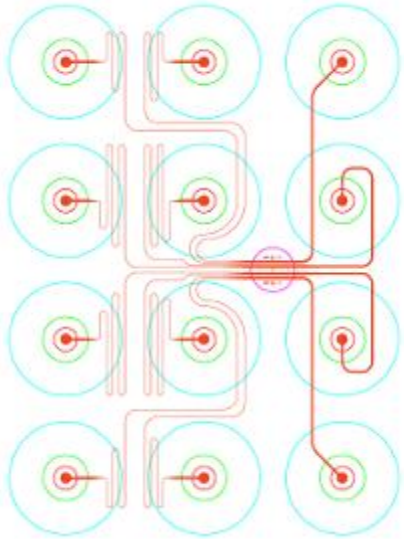


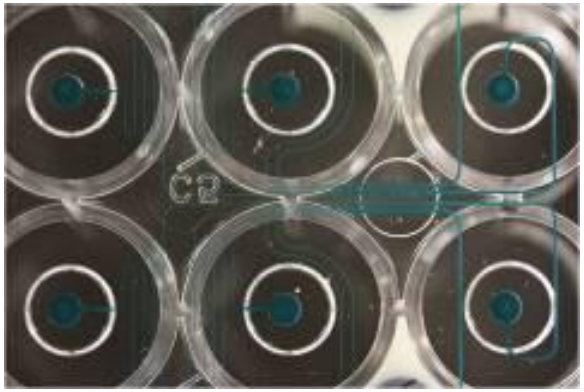
BioFlux Plates – Tailored for Cell Assays

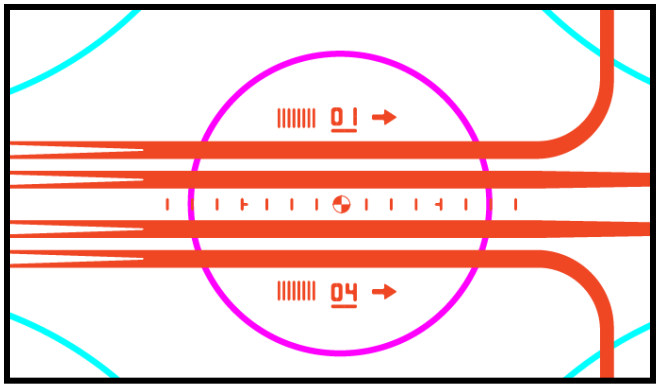
- SBS well plate formats for convenience
- 180µm (#1.5) cover glass bottoms for optimal imaging
- Micron-scale fluidics for minimal sample volume requirements
  - Viewing channels are 70µm tall X 350µm wide
- Channels are produced using an extremely accurate manufacturing technique which contributes to data reliability and accuracy
- Innovative pneumatic format allows all experiments on a plate to run simultaneously without additional pumps
- Multiple plate formats to suit a wide range of application requirements

BioFlux 24-well Plate

0-20 dyn/cm^2







24-well BioFlux Plate

0-20 dyne/cm2

8 experimental channels

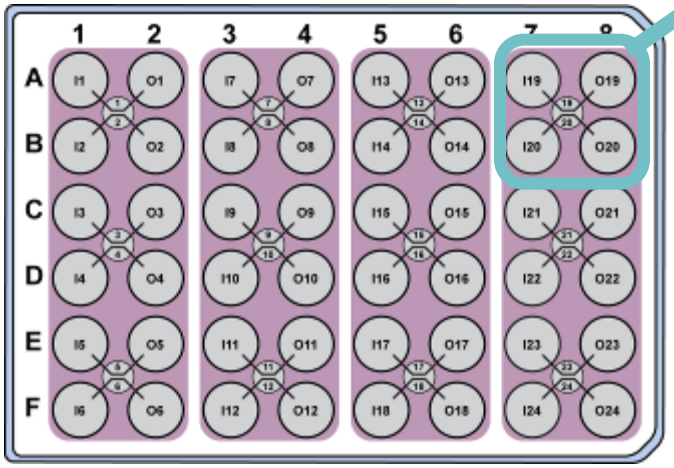
2 input wells per channel/3 ml per well

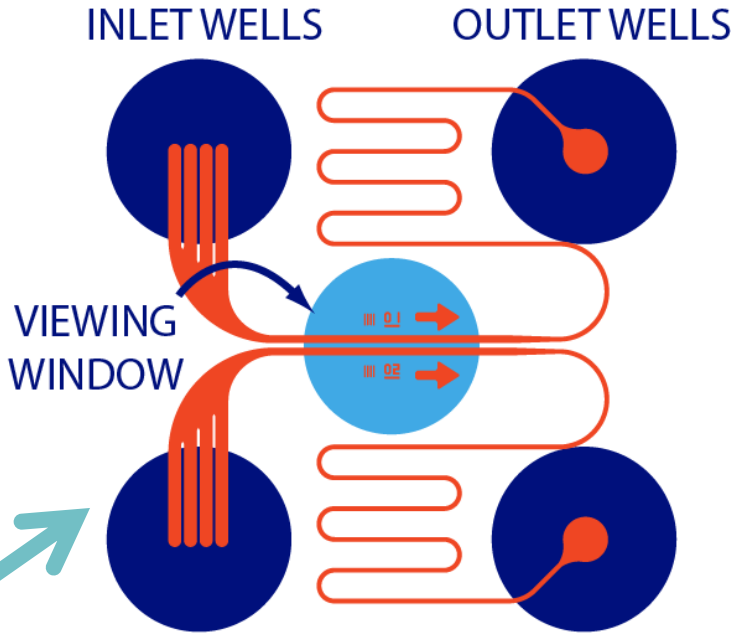
Dynamic switching of media, compounds, wash buffers, etc. between input wells

or parallel flow

BioFlux 48-well Plate

0-20 or 0-200 dyne/cm^2





48-well BioFlux Plate

0-20 dyne/cm^2

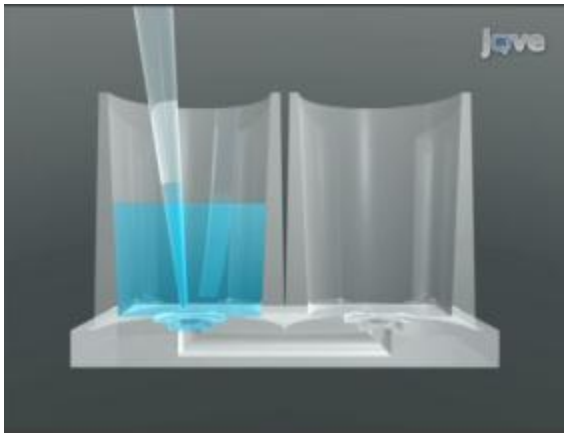
0-200 dyne/cm^2

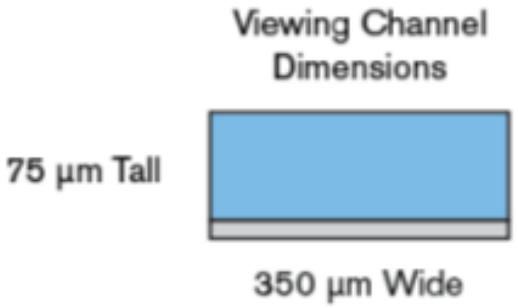
24 experimental channels

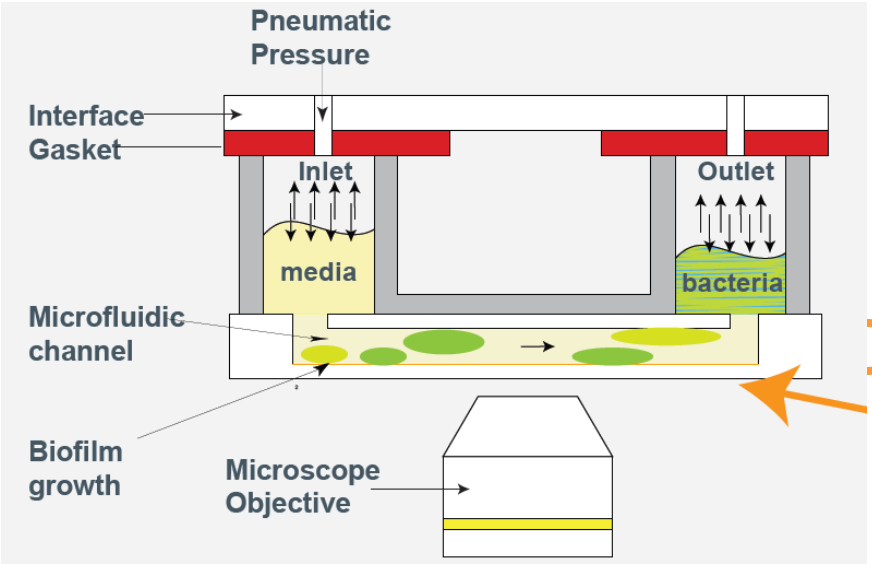
1 input well per channel

High shear range for platelet studies, adhesion, compound screening

Principles of Operation







polystyrene well plate

PDMS

glass,180 µm

Selected BioFlux Applications

Inflammation/Vascular/ Cancer Biology

Platelet adhesion
Endothelial cell biology
Immune cell adhesion
Rolling adhesion
Transmigration
Chemotactic Migration
Metastasis/3D invasion
Angiogenesis

General Cell-Based Assays

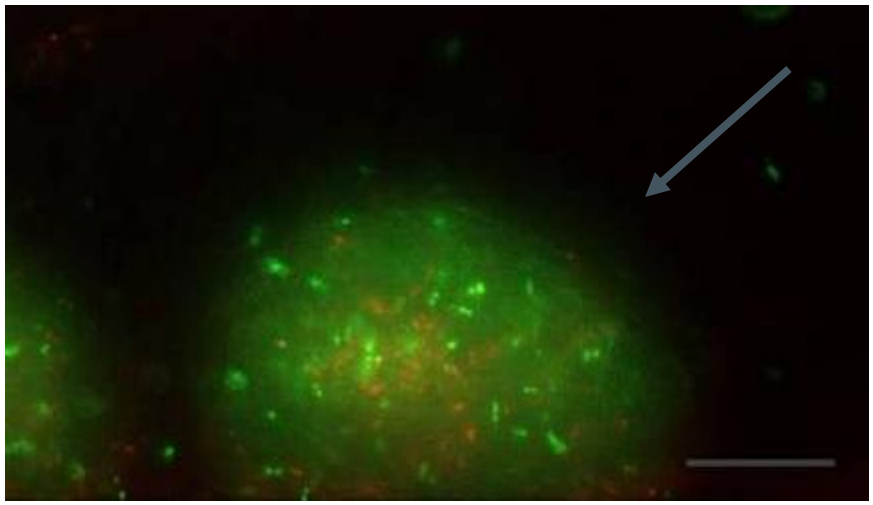
Stem cell differentiation
Neurite outgrowth
Renal cell biology
Wound healing
Ca^{2+} release

Microbiology

Microbial biofilms
Host pathogen interactions
Microbial adhesion
Parasitology

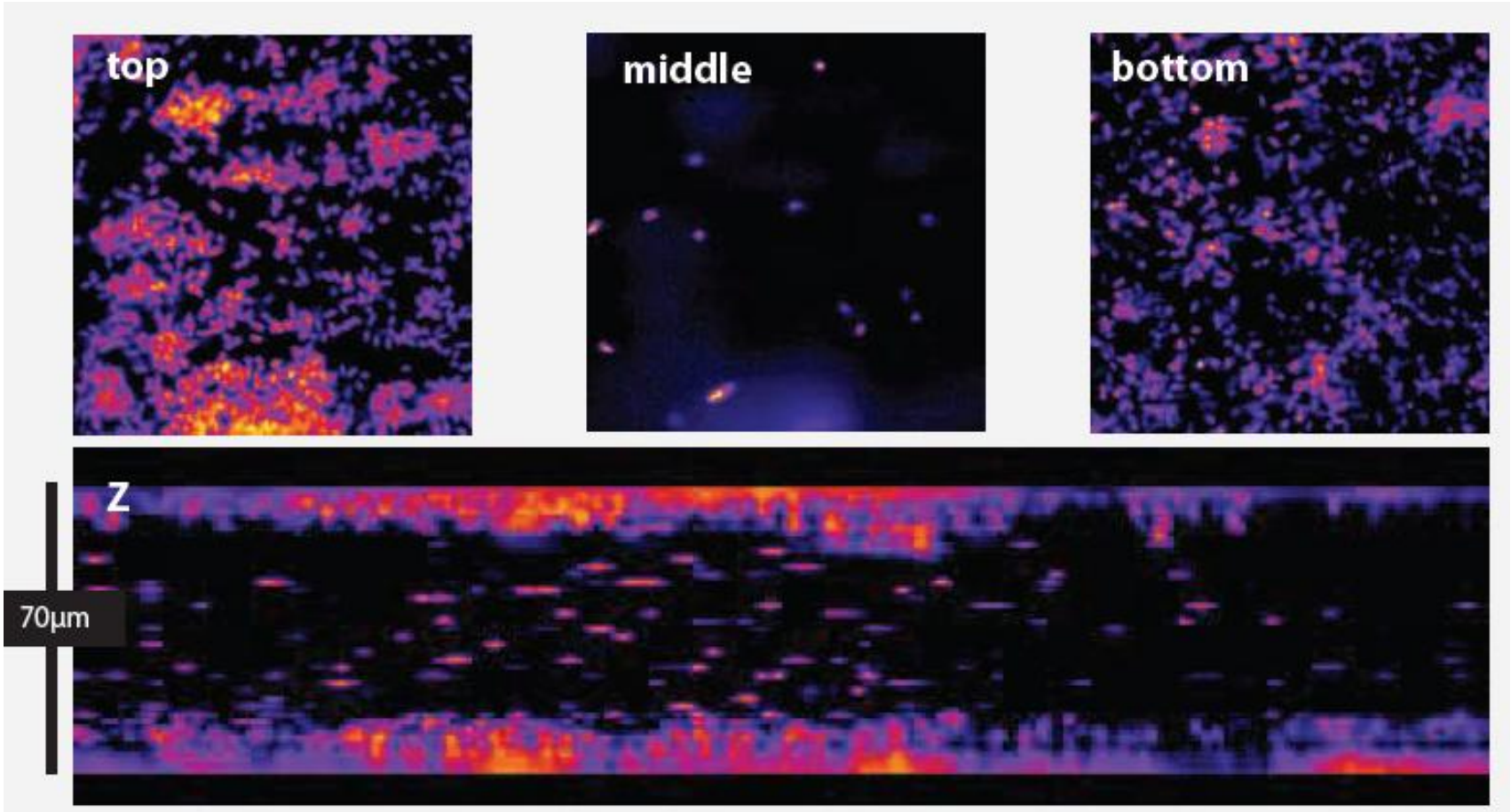
Why Flow-Based Microfluidics for Biofilms?

- **Parallelization with very low reagent usage**
- High content data
- Control over micro/environment
- Continuous and bolus perfusion of compounds
- View biofilm 3D architecture
- View the effect of compounds in *in situ* context



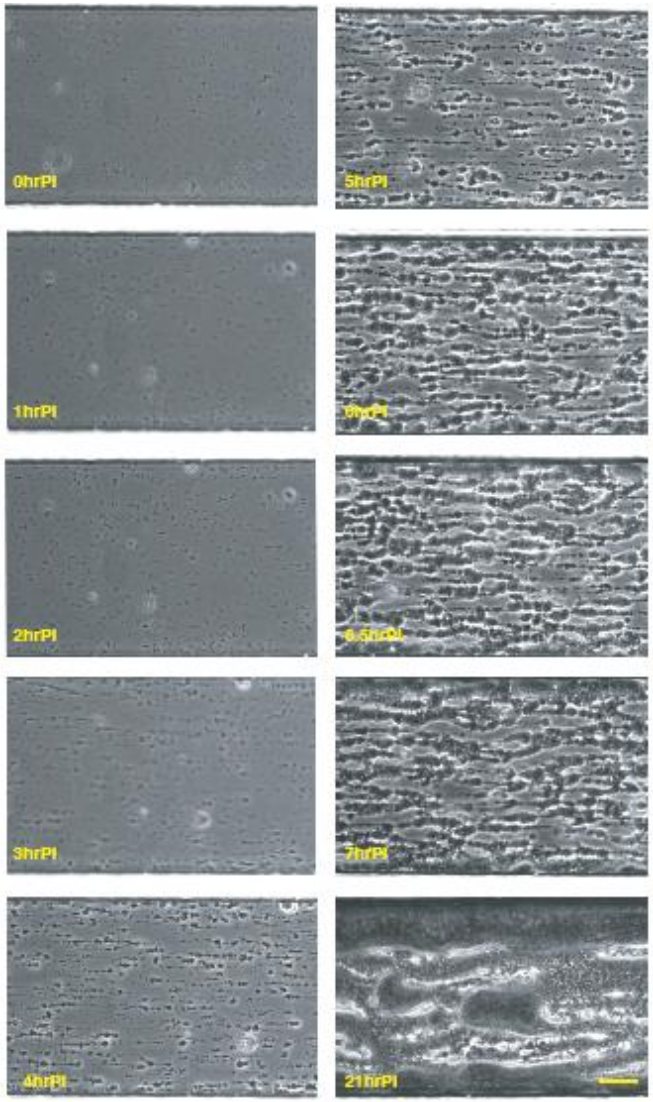
***P. aeruginosa* microcolony in microfluidic channel. Scale bar 40µm**

Model Biofilm Growth Inside the Bioflux Channels

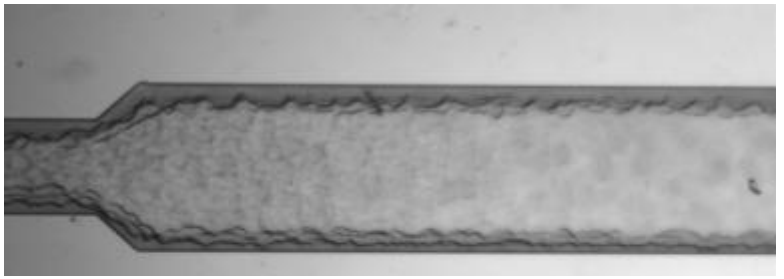


Pseudomonas fluorescens, 24 hrs post inoculation, imaged under flow, swept field confocal, Nikon center, UCSF

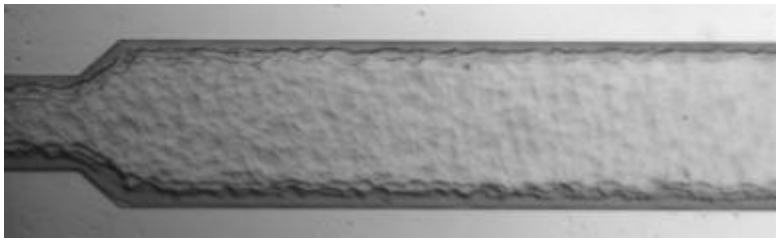
'Simple' Biofilms Within the Microfluidic Channels

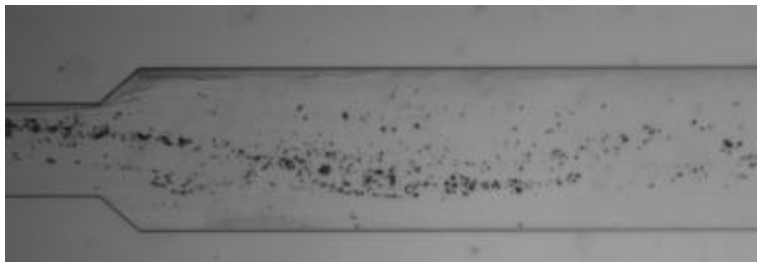


***P. fluorescens*, TSB, 21 hrs, 2dyn/cm²**



***P. aeruginosa* PA01, LB, 16 hours, 1dyn/cm1dyn/cm²**

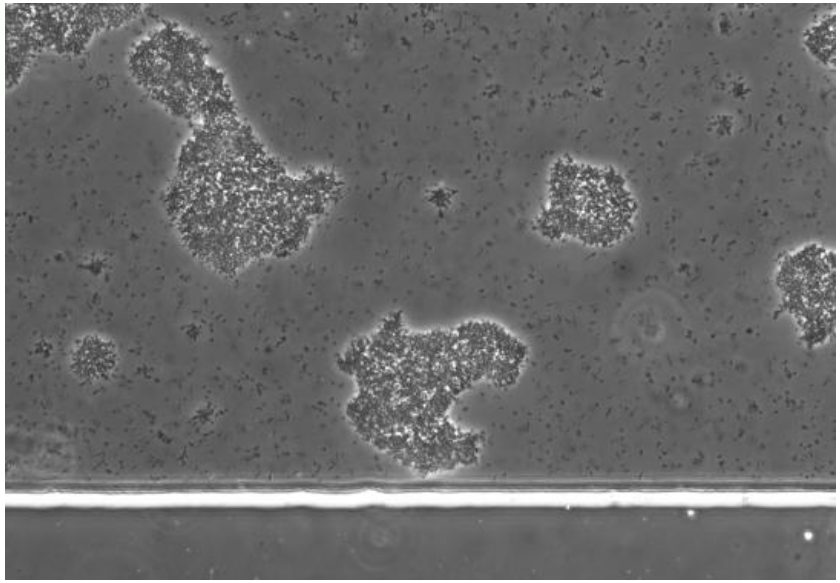




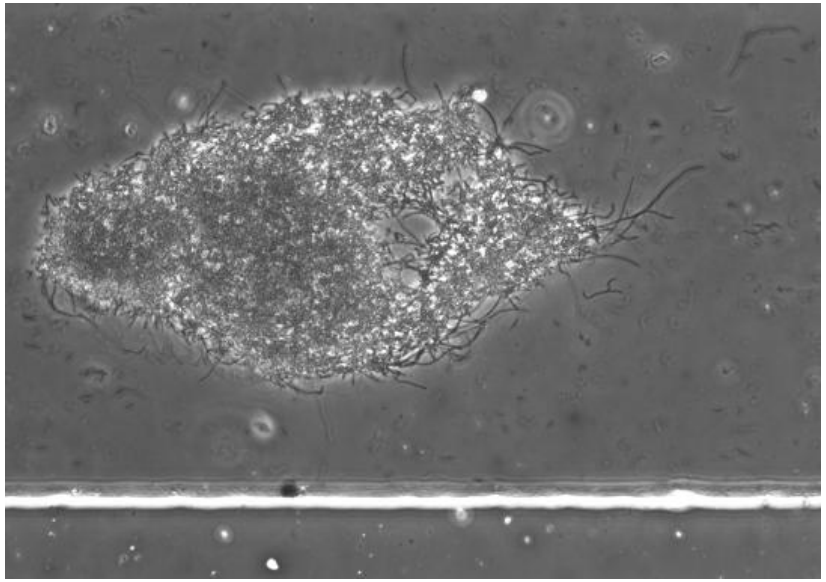
***P. aeruginosa* PA01, disrupted, 1dyn/cm1dyn/cm²**

Growth of Biofilms on Adherent Substrates

***B. subtilis* on Cell-tak™**



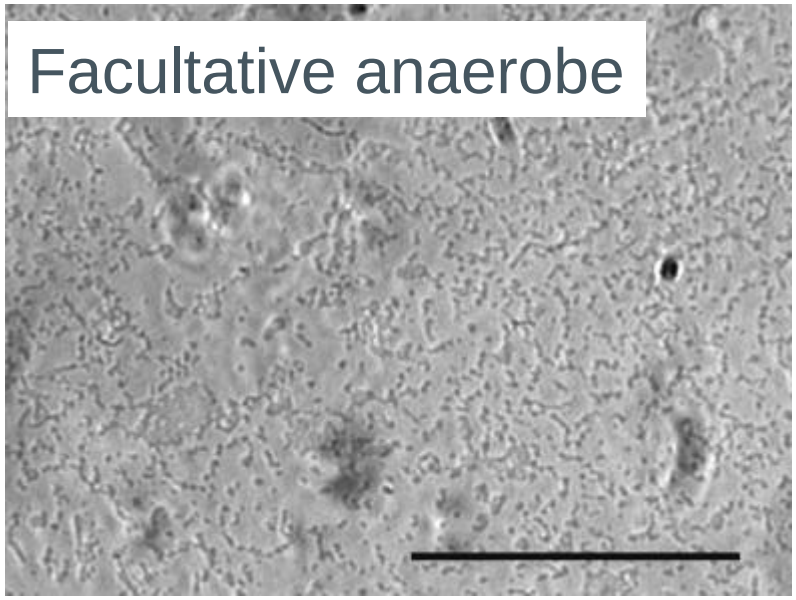
0.4 dyn/cm^2



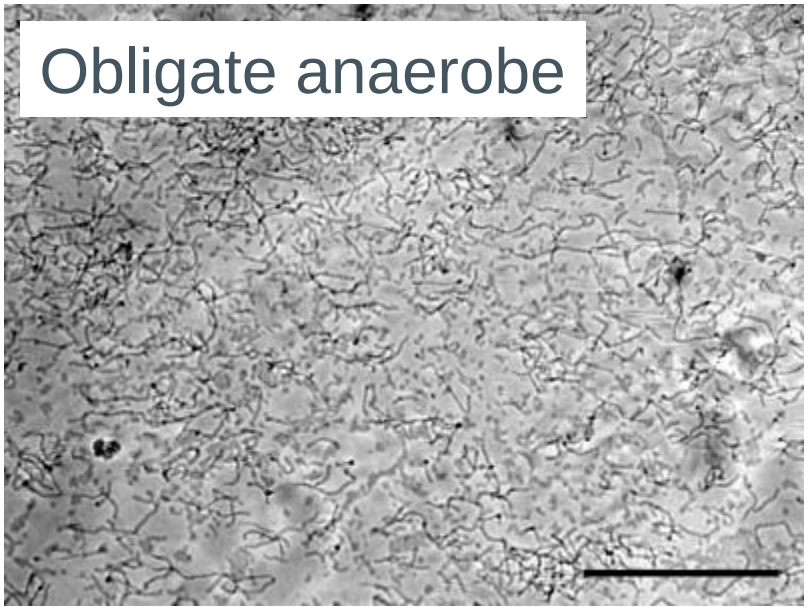
1dyn/cm^2

Specialized Microenvironments – Anaerobic

5%CO_2 : 95% nitrogen gas mix



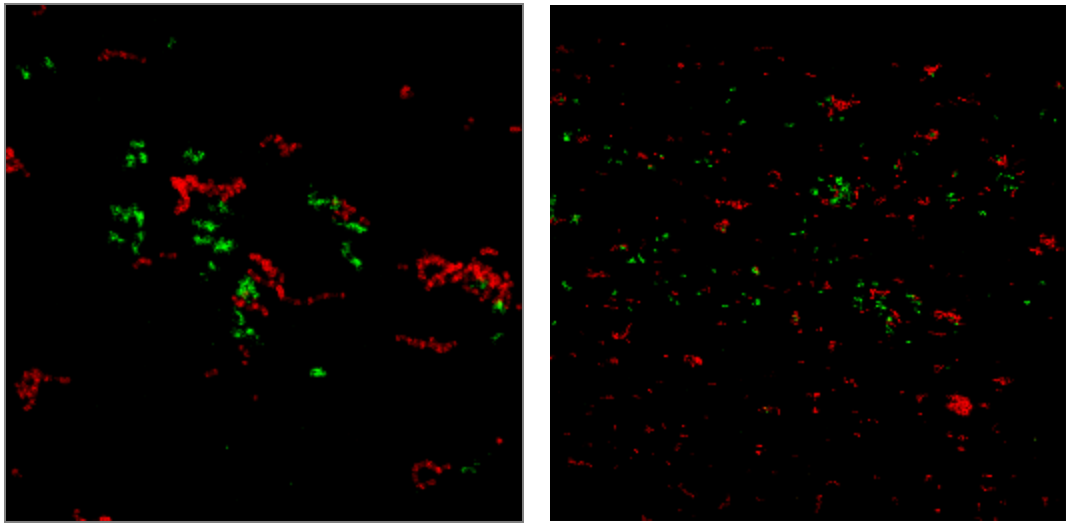
S. gordonii, 24hours



S. gordonii + P. gingivalis, 6 hours

Mixed Species Biofilms

Co-culture of *Streptococcus oralis* and *Actinomyces naeslundii* in the BioFlux system for examination of cooperative/ mutualistic biofilm formation



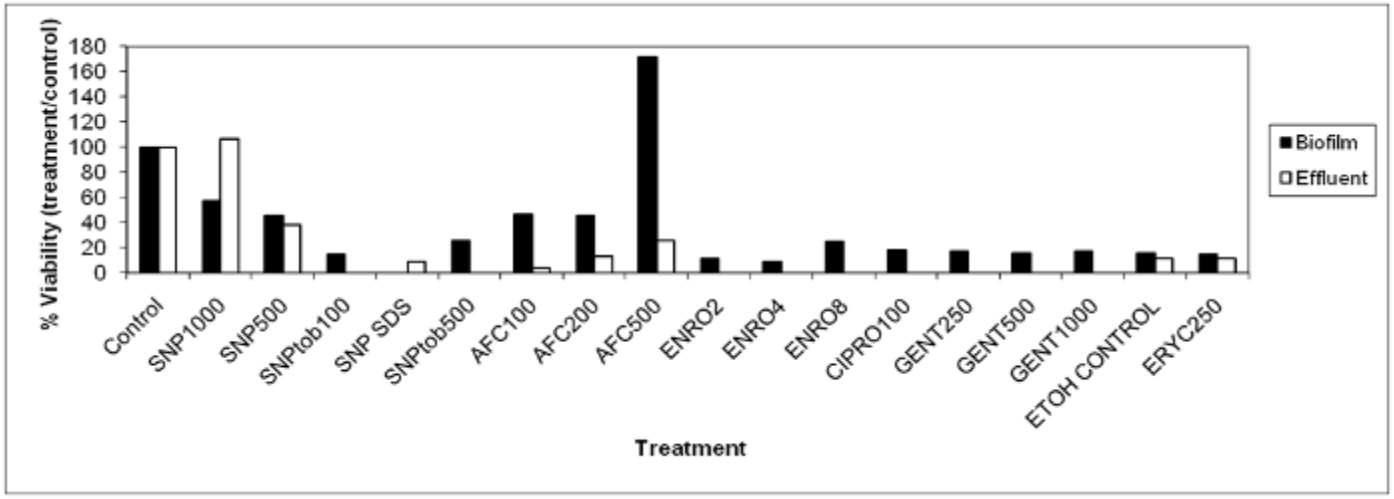
Oral biofilms grown on saliva, Co-aggregation dependent biofilm formation,
Stained with anti-organism antibodies

Collaboration with Robert Palmer, Albert Ding, NIH

Antimicrobial Compound Profiling

Channel	Drug	[Conc]	Mode of action	Reference
1	Control			
2	Sodium Nitroprusside (SNP)	1000nM	Nitric oxide pathway dispersal of biofilms (sublethal conc)	Barraud et al 2006 J. Bac p7344
4	SNP	500nM		Barraud et al 2006 J. Bac p7344
5	SNP+tobramycin (tob)	500nM 100uM	tob inhibits 30S, kills only planktonic cells	Barraud et al 2006 J. Bac p7344
6	SNP+SDS	500nM 0.05%	SDS- cell lysis	Barraud et al 2006 J. Bac p7344
7	SNP+tob	500nM 500uM		Barraud et al 2006 J. Bac p7344
8	Ferric ammonium citrate (AFC)	100 uM	repress gene	Musk et al 2005 Chem&Biol p789
9	AFC	200 uM	expression for	Musk et al 2005 Chem&Biol p789
10	AFC	500 uM	BF formation	Musk et al 2005 Chem&Biol p789
11	Enrofloxacin (Enro)	2 ug/ml	binds gyrase	Olson et al 2002 Can J Vet Res p86
12	Enro	4ug/ml	antibiotic	Olson et al 2002 Can J Vet Res p86
13	Enro	8ug/ml		Olson et al 2002 Can J Vet Res p86
14	Ciprofloxacin (Cipro)	5ug/ml	binds gyrase	Reid et al 1994 AAC p1490
15	Cipro	50ug/ml	antibiotic	Reid et al 1994 AAC p1490
16	Cipro	100ug/ml		Reid et al 1994 AAC p1490
17	Gentamicin (Gent)	256 ug/ml	30S ribo/trans	Olson et al 2002 Can J Vet Res p86
18	Gent	512 ug/ml	antibiotic	Olson et al 2002 Can J Vet Res p86
19	Gent	1024 ug/ml		Olson et al 2002 Can J Vet Res p86
20	control (10%ethanol)			
22	Erythromycin (Eryc)	64 ug/ml	50S ribo/ trans	Olson et al 2002 Can J Vet Res p86
23	Eryc	256 ug/ml	antibiotic	Olson et al 2002 Can J Vet Res p86
24	Eryc	512 ug/ml		Olson et al 2002 Can J Vet Res p86

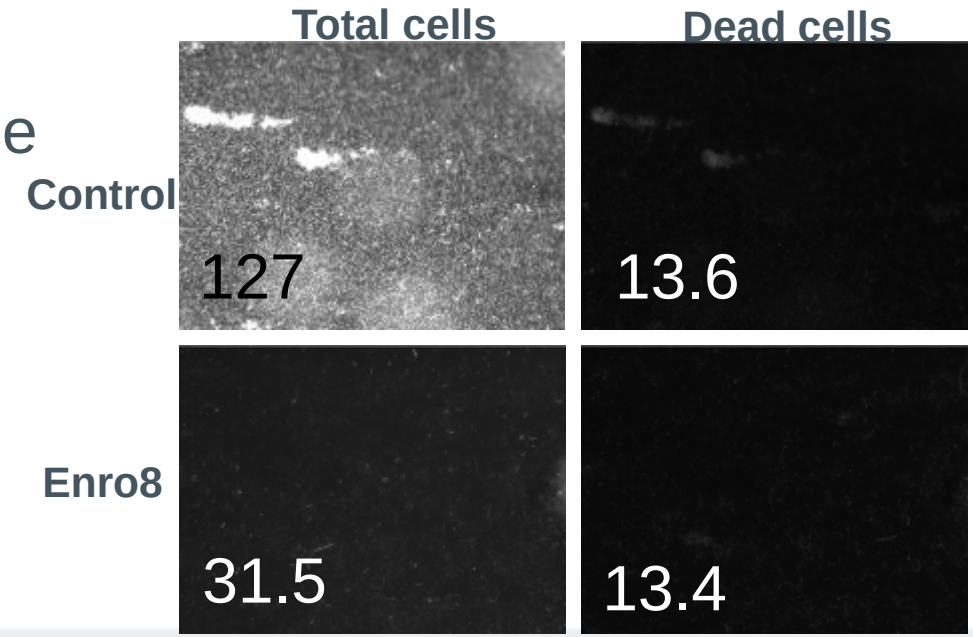
Antimicrobial Compound Profiling



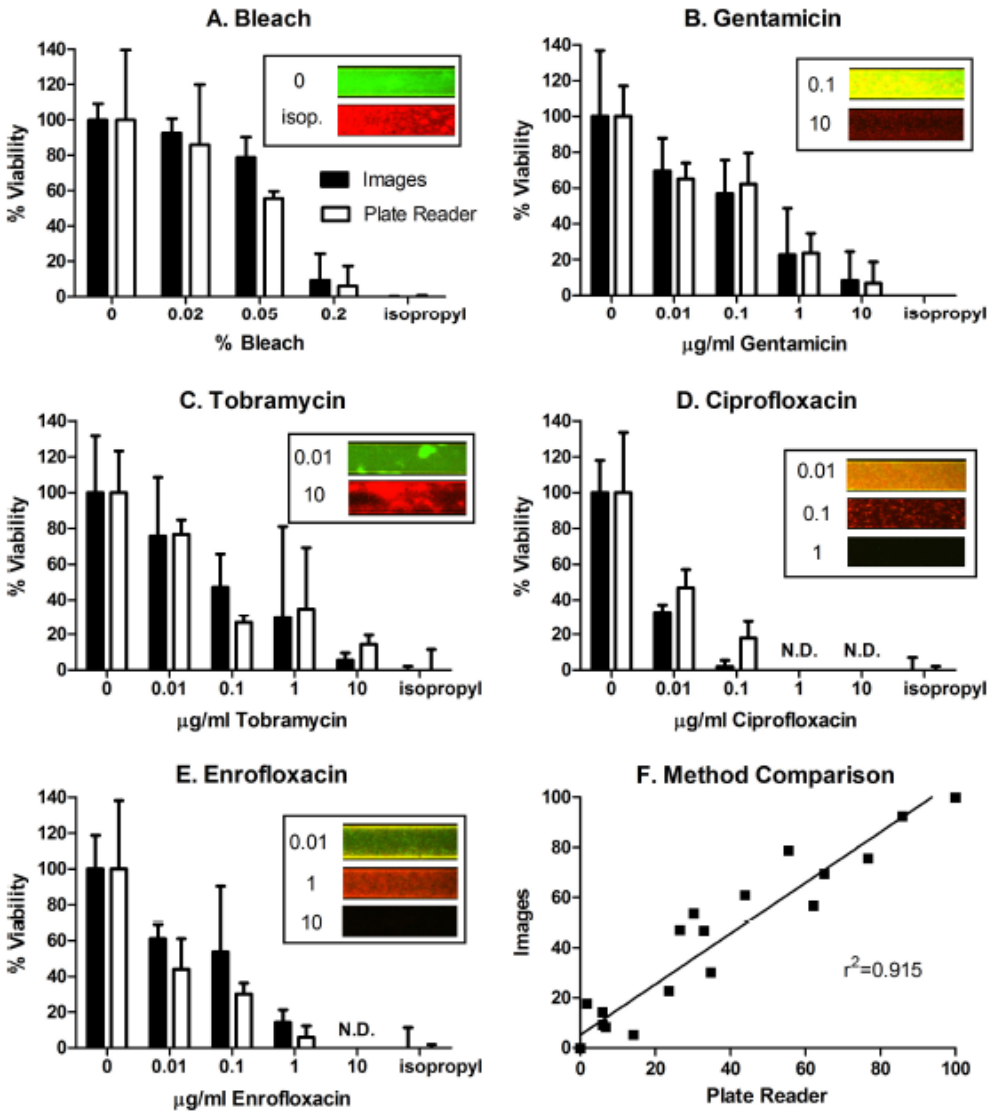
2 Data Streams:

- Live-Dead Fluorescence stain intensity
- CFU in Effluent

96 biofilms can be grown at once under identical conditions.



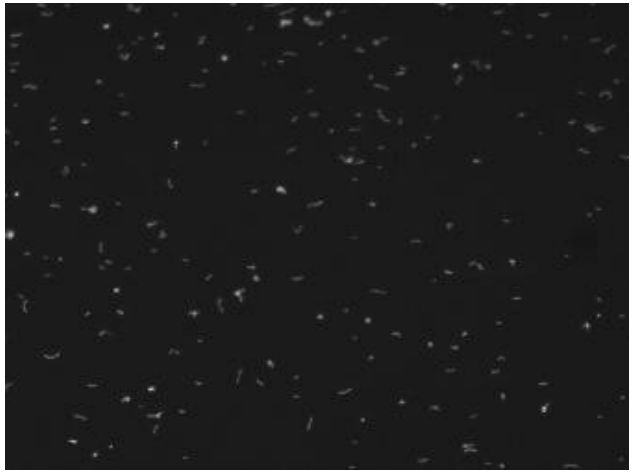
Viability Screening of *P. aeruginosa* - Biofilms HT



Benoit et al. 2010 AEM 76:4136

Microbial Adhesion Strength Under Mechanical Loads

Study force needed to displace bacteria on different substrata or chemical mediators for displacement or adhesion kinetics

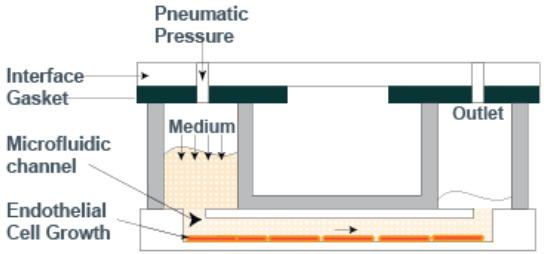


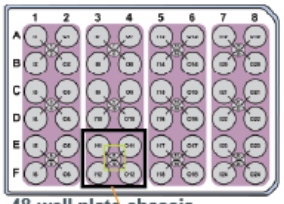
	Adhesion 1 dyn/cm2	Adhesion 20 dyn/cm2
Pathogen **WT** gene	100%	100%
Pathogen **Mut** adhesion gene	25.1%	10%
Supplemented Pathogen **WT** adhesion gene	100%	100%
Parental *E.coli*	83%	5%

Host-Pathogen Interactions

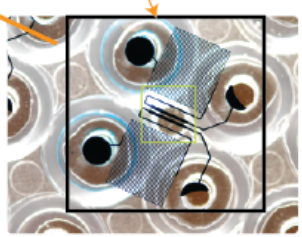
FLUXION

1. Seed and grow endothelial cell monolayers



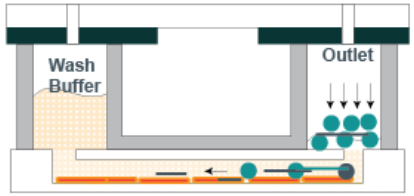


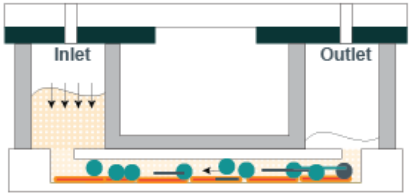
48-well plate chassis



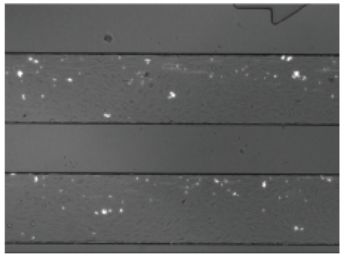
Microfluidic channels connected to wells. Wells are reservoirs for media and cells.

2. Add *C. albicans* cells to the monolayer under shear flow conditions



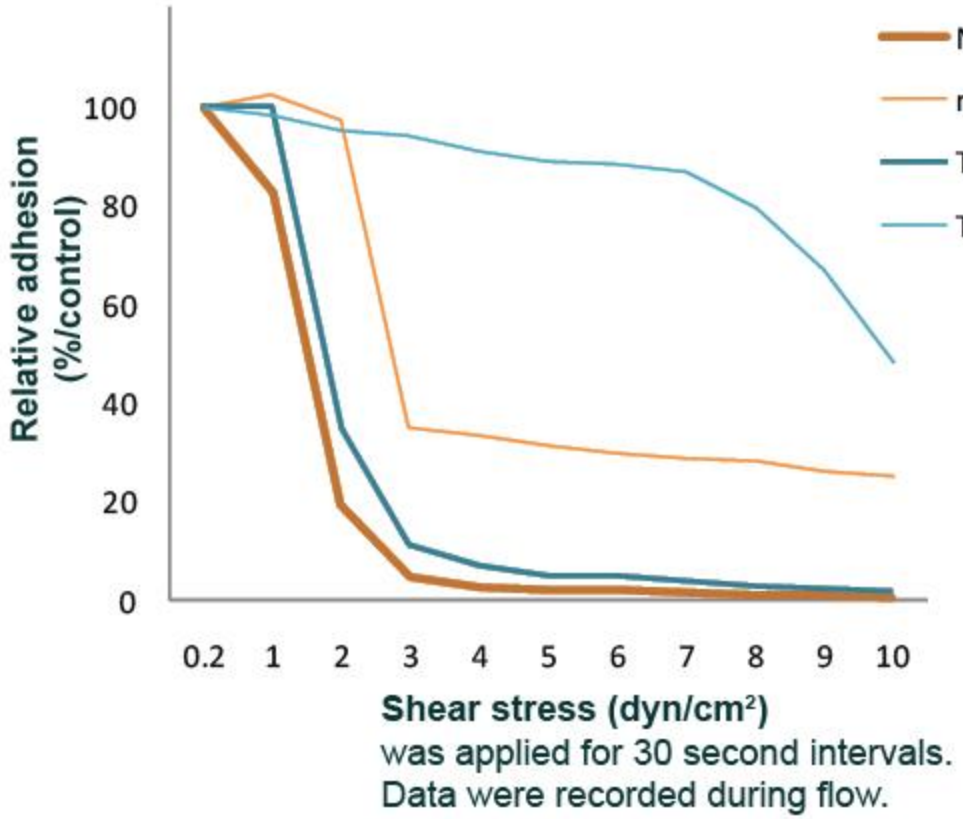


3. Switch direction of flow to wash away unbound cells



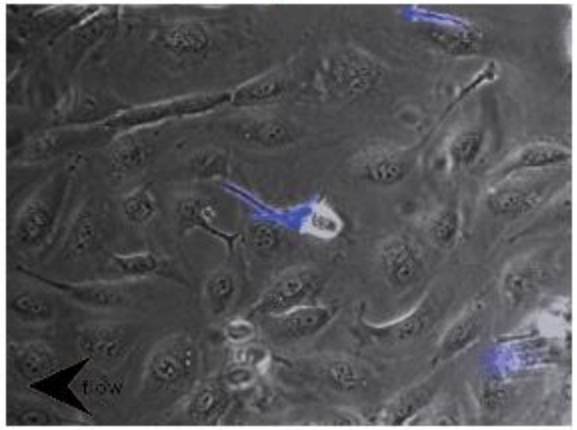
4. Observe using inverted microscopy and timelapse imaging.

C. albicans Adhesion Strength to Endothelial Cell Monolayers

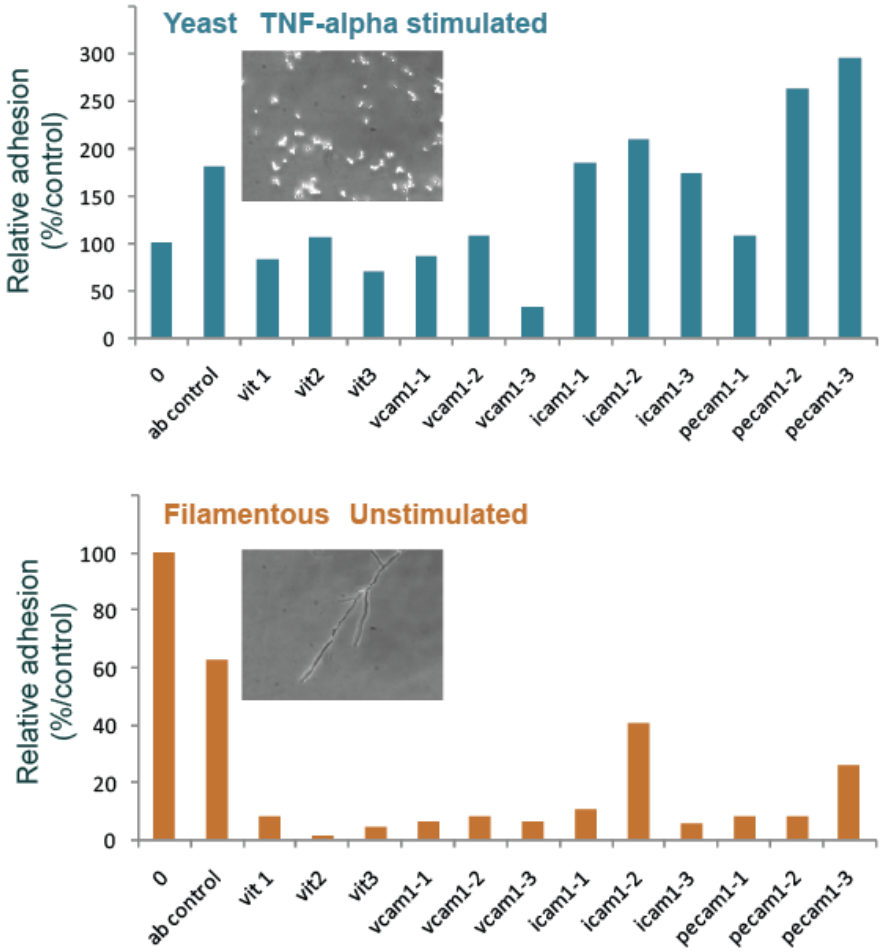


was applied for 30 second intervals. Data were recorded during flow.

Cells attached to the monolayers under low flow conditions (0.2 dyn/cm²) Under conditions of physiological shear (0-10 dyn/cm²), we found that the filamentous forms of this strain adhered very tightly to the monolayers when compared to the yeast forms. Both forms resisted shear up to 2 dyn/cm². **However with filamentous forms under conditions of inflammation resistance to shear exceeded 20dyn/cm².**



Inhibition of *C. albicans* Adhesion to Endothelial Cell Monolayers



Host- Pathogen Interactions: *P. aeruginosa* and Calu-3 cells

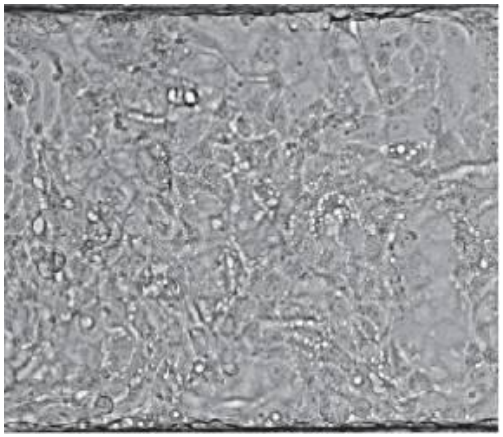


Figure1: Airway epithelial cells grown to 100% confluence in the BioFlux plate (scale bar = 100µm).

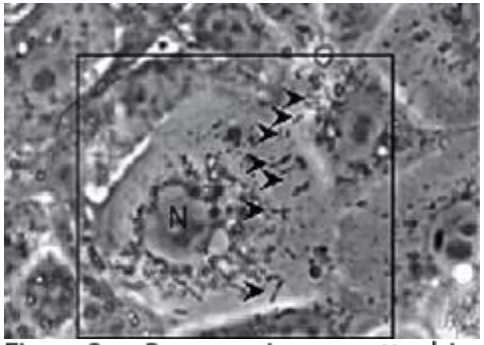


Figure2: *P. aeruginosa* attaching under flow to airway epithelial cells (scale bars=20µm).

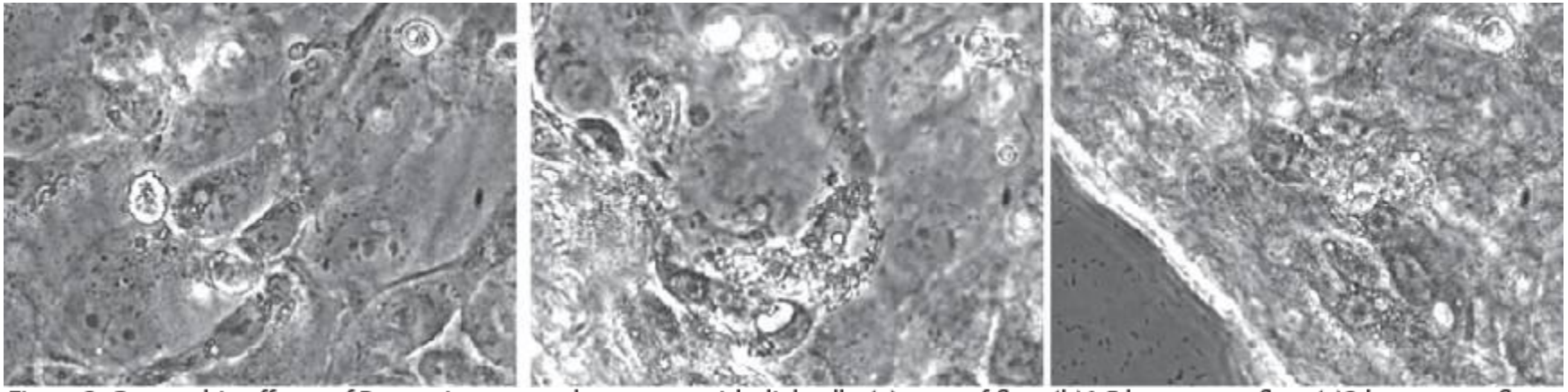
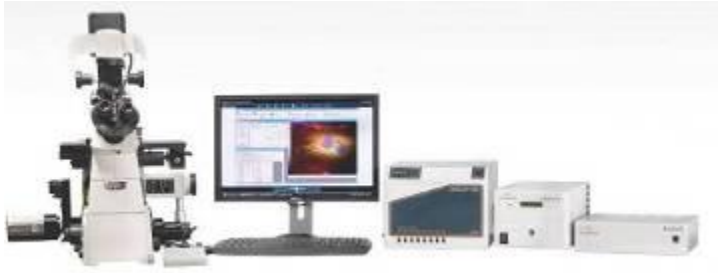


Figure3: Cytopathic effects of P.aeruginosa attachment to epithelial cells. (a) start of flow (b)1.5 hours post flow (c)3 hours post flow (scale bar= 40 µm)

Key Benefits for Dynamic Live-cell Assays

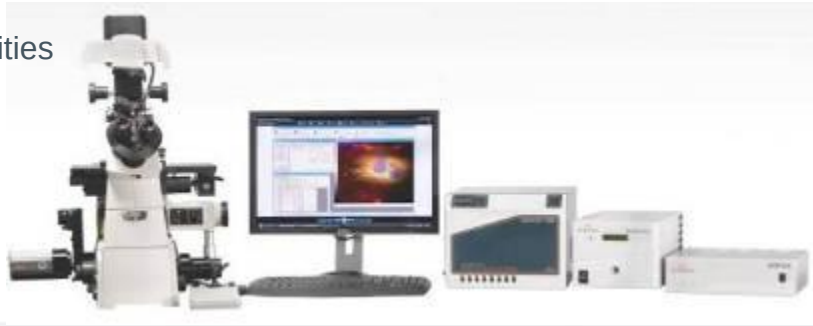
- Flexible format enables a wider range of assays
- Higher quality/content data than transwell membrane assays
- Real-time measurements *in situ*
- Minimizes variability with a continuous gradient
- Saves time by eliminating transfer steps normally required with transwell assays
- Assays can be performed label-free
- Physiological shear flow



BioFlux Configuration Options

- BioFlux 200 System
  - Integrates with any inverted microscope
  - Contains:
    - Pneumatic Controller
    - Heating Plate
    - Pressure Interface
    - BioFlux 200 Control and acquisition software

- BioFlux 1000 and 1000Z Systems
  - Fully integrated system for automated operation
  - Ideal for time lapse observation, drug screening, high throughput use, core labs
  - Contains:
    - Pneumatic Controller
    - Heating Plate
    - Pressure Interface
    - BioFlux Montage Software with full analysis capabilities
    - Automated inverted microscope
    - Multi-wavelength fluorescence acquisition



Conclusion

- For additional information on Applications and Product Details, visit our website: www.fluxionbio.com
- For a sales quotation or ordering information
  - Email: sales@fluxionbio.com
  - Phone: (866) 266-8380
- Westburg – Fluxion Benelux & Scandinavia: www.westburg.eu